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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte SHUYUAN ZHANG, CAPUCINE THWIN,
ZHENG WU, TOOHYON CHO, and
SHAWN GALLAGHER

Appeal 2009-002156[1]
Application 09/203,078
Technology Center 1600

Decided: August 13, 2009

Before FRANCISCO C. PRATS, MELANIE L. McCOLLUM, and JEFFREY N. FREDMAN, *Administrative Patent Judges*.

FREDMAN, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a process for preparing adenovirus. We have jurisdiction under 35 U.S.C. § 6(b). We affirm-in-part.

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[1] Oral Hearing held July 21, 2009.

Statement of the Case

Background

"As the clinical trials progress, the demand for clinical grade adenoviral vectors is increasing dramatically. The projected annual demand for a 300 patient clinical trial could reach approximately 1.08×10^{16} viral particles" (Spec. 2, ll. 18-20). According to the Specification, "[t]raditionally, adenoviruses are produced in commercially available tissue culture flasks, 'cellfactories,' or RB." (Spec. 2, ll. 22-23.) The Specification states that "in order to achieve large scale adenoviral vector production, purification methods other than CsCl gradient ultracentrifugation have to be developed" (Spec. 3, ll. 6-7).

The Claims

Claims 1-4, 8-31, and 38-62 are on appeal. We will focus on claims 1-4, 8, 9, 13, 29, and 47, which are representative and read as follows:

> 1. A process for preparing adenovirus, the process comprising:
>
> (a) preparing a culture of producer cells in a selected media;
>
> (b) infecting producer cells in the culture with the adenovirus, wherein the producer cells are infected between mid-log phase of growth and stationary phase of growth; and
>
> (c) harvesting adenovirus from the cell culture.
>
> 2. The process of claim [1 wherein infecting producer cells in the culture with the adenovirus occurs in a bioreactor system, a microcarrier culture system, a multiplate culture system, a perfused packed bed reactor system, or a microencapsulation culture system]
> wherein the producer cells are infected with the adenovirus between late-log phase and stationary phase of growth.

3. The process of claim 1, wherein the producer cells are essentially homogeneous with respect to the phase of cell growth.

4. The process of claim 1, wherein the producer cells are perfused for at least a portion of the time that the cells are cultured.

8. The process of claim 1, wherein the producer cells are seeded into the culture medium and allowed to attach to a culture surface for between about 3 hours and about 24 hours prior to infection with adenovirus.

9. The process of claim 1, wherein the culture medium is at least partially recirculated during the adenovirus infection step.

13. The process of claim 1, wherein the harvested adenovirus is subjected to purification and placed into a pharmaceutically acceptable composition.

29. The process of claim 1, further comprising purifying the harvested adenovirus to obtain a purified adenovirus composition having one or more of the following properties:

(a) a virus titer of between about 1 x 109 and about 1 x 1013 pfu/ml;

(b) a virus particle concentration between about 1 x 1010 and about 2 x 1013 particles/ml;

(c) a particle:pfu ratio between about 10 and about 60;

(d) having less than 50 ng BSA per 1 x 10^{e12} viral particles;

(e) between about 50 pg and 1 ng of contaminating human DNA per 1 x 1012 viral particles,

(f) a single HPLC elution peak consisting essentially of 97 to 99% of the area under the peak.

47. In a method for producing adenovirus that includes culturing producer cells and infecting the cultured producer

cells with an adenovirus, wherein the improvement comprises infecting said producer cells with the adenovirus when the cells in culture are between mid-log phase of growth and stationary phase of growth.

The prior art

The Examiner relies on the following references to show unpatentability:

Scheer	US 5,106,841	Apr. 21, 1992
Leu et al	US 6,194,210 B1	Feb. 27, 2001

Huyghe et al., *Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 by Column Chromatography*, 6 HUMAN GENE THERAPY 1403-1416 (1995).

Garnier et al., *Scale-up of the adenovirus expression system for the production of recombinant protein in human 293S cells*, 15 CYTOTECHNOLOGY 145-155 (1994).

Kuchler R.J., In *Biochemical Methods in Cell Culture and Virology*, Stroudsburg, Penn: Dowden, Hutchingson & Ross, Inc., 90, 91, 99, 100, (1977).

Graham et al., In *Methods in Molecular Biology: Gene Transfer and Expression Protocols* 7. Murray, E.J. Editors. Clifton, NJ: Humana Pres, 109-120 and 205-225 (1991).

Spier, R.E. and J.B. Griffiths, eds., 3 ANIMAL CELL BIOTECHNOLOGY 179-219, (1988).

Mediatech Techical Information Bulletin (No citation information available - - provided by Appellant as an attachment to an affidavit submitted February 28, 2002).

Murphy et al., *Virus Taxonomy*. In B.N. Fields et al. (ed.), FIELDS VIROLOGY, 3rd ed. Philadelphia: Lippencott-Raven Publishers; Table 6, pages 51-54 (1996).

The issues

A. The Examiner rejected claims 1, 3, 8, 9, 13-25, 31, 38, 47, 49, and 51-62 under 35 U.S.C. § 102(b) as being anticipated by Huyghe in light of Kuchler.

B. The Examiner rejected claims 10-12 and 29 under 35 U.S.C. § 103(a) as being obvious over Huyghe.

C. The Examiner rejected claims 2 and 50 under 35 U.S.C. § 103(a) as being obvious over Huyghe, Graham and Leu.

D. The Examiner rejected claims 26-28 under 35 U.S.C. § 103(a) as being obvious over Huyghe and Graham.

E. The Examiner rejected claims 4, 30, 39-46, and 48 under 35 U.S.C. § 103(a) as being obvious over Huyghe, Garnier and Spier.

A. 35 U.S.C. § 102(b) over Huyghe

The Examiner finds that "Huyghe et al. anticipate preparing adenovirus by preparing a culture of 293 producer cells that have a attained an essentially homogenous confluency of 50-60% when the cells are infected with a replication-defective adenovirus expressing p53 from a CMV promoter in place of E1 coding sequences" (Ans. 5-6). The Examiner finds that "[t]his percentage of confluency reasonably corresponds to mid-log phase of cell growth" (Ans. 6).

Appellants contend that the "Examiner has not met the burden of proof to establish inherent anticipation of independent claims 1 and 47 by Huyghe in light of Kuchler under 35 U.S.C. § 102(b) because the Examiner has neither shown that the characteristics of the present invention are necessarily present in the prior art, nor provided a basis to reasonably

support such a determination" (App. Br. 7). Appellants also contend that Huyghe does not anticipate claims 3, 8, 9, 13, 31, and 58 (App. Br. 14-17).

In view of these conflicting positions, we frame the anticipation issues before us as follows:

(i) Did the Examiner err in finding that Huyghe teaches "infecting producer cells in the culture with the adenovirus, wherein the producer cells are infected between mid-log phase of growth and stationary phase of growth" as required by claim 1?

(ii) Did the Examiner err in finding that Huyghe teaches that "the producer cells are essentially homogeneous with respect to the phase of cell growth" as required by claims 3 and 58?

(iii) Did the Examiner err in finding that Huyghe teaches that the producer cells are "allowed to attach to a culture surface for between about 3 hours and about 24 hours prior to infection with adenovirus" as required by claim 8?

(iv) Did the Examiner err in finding that Huyghe teaches that "the culture medium is at least partially recirculated during the adenovirus infection step" as required by claim 9?

(v) Did the Examiner err in finding that Huyghe teaches "a pharmaceutically acceptable composition" as required by claims 13 and 31?

Findings of Fact (FF)

1. The Specification teaches that the

> log phase of the growth curve is where the cells reach their maximum rate of cell division (*i.e.* growth). The term mid-log phase of growth refers to the transition mid-point of a logarithmic growth curve. Stationary phase growth refers to the time on a growth curve (*i.e.*

> a plateau) in which cell growth and cell death have come to equilibrium.

(Spec. 5, ll. 1-5.)

2. The Specification teaches that "mid log phase can be approximately defined as the point or interval within the log phase in which the cells are dividing at their maximal rate, and the increase in logs of cell number is linear with respect to time." (Spec. 22, ll. 14-17.)

3. The Specification distinguishes "mid-log phase" from "[l]ate log phase," which is the "point or interval of time in which the rate of cell division has slowed" (Spec. 22, ll. 17-18).

4. The Specification teaches that 293 cells had a doubling time of "about 1.3 day which is comparable to 1.2 day of the cells in 10% FBS media in the attached cell culture" (Spec. 142, ll. 12-13).

5. The Specification teaches that the "the cells were infected at 5 MOI when the cells reached 1.36E+06 viable cells/mL on day 3" (Spec. 144, ll. 7-8).

6. The Specification teaches that

> [h]igher numbers of cells used in the cell inoculation step results in a cell density that is too high and the result is an over-confluence of cells at the time of viral infection, thus lowering yields. It is well within one of skill in the art to determine that in other types of cell culturing systems, similar optimization of the seeding density for a particular system could easily be determined.

(Spec. 164, ll. 9-14.)

7. Huyghe teaches that "293 cells . . . were grown in a 6,000-cm^2 Cell Factory (Nunc) in a humidified air/7% CO_2 incubator" (Huyghe 1404, col. 1).

8. Huyghe teaches that "[t]wo to 2.5 days after seeding the Cell Factory, when cell monolayers reached about 50-60% confluency, the cells were infected at a multiplicity of infection (moi) of 5-10 infectious units (IU) per cell in 500 ml of fresh medium" (Huyghe 1404, col. 1).

9. Huyghe teaches that "[t]hree to 4 days post-infection, the infected cells were ready for harvesting" (Huyghe 1404, col. 1). Huyghe teaches that "the cells were harvested by gentle tapping and centrifuged in a Beckman TJ-6" (Huyghe 1404, col. 1).

10. Huyghe teaches that the "final virus pool from this step was dialyzed extensively against phosphate-buffered saline (PBS) supplemented with 2% sucrose and 2 m*M* $MgCl_2$" (Huyghe 1404, col. 2).

11. Kuchler teaches that the

> lag phase usually varies from 24 to 48 hours . . . When the cells begin to divide, the population enters the logarithmic phase of the culture cycle in which the cell number is increasing at a constant rate. During this period, which lasts 2 to 8 days, the population at any time is composed of cells that are at all points in the division cycle.

(Kuchler 90.)

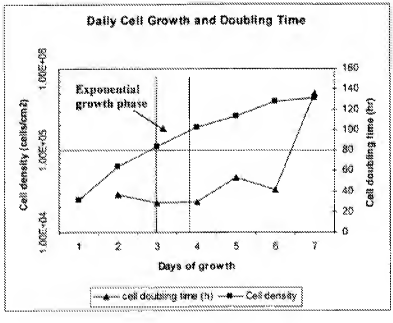
12. Kuchler teaches that the "population doubling time for cultured cells ranges from 12 to 48 hours. Since cultures are usually started with 50,000 to 200,000 cells, four or five population doublings occur during the culture cycle" (Kuchler 90-91).

13. Mediatech teaches that "[t]hese fresh cultures are allowed to grow and divide as normal until such time the culture reaches confluence and the cells are used for experiments or subcultured" (Mediatech, subheading "Cell Dissociation").

14. The Zhang Declaration teaches that "in studies conducted at Introgen Therapeutics involving the growth of 293 cells in T-150 flasks, a cell doubling time of approximately 30 hours during exponential growth phase was observed" (Zhang Dec. September 2004 ¶ 6).

15. The Zhang Declaration teaches that for frozen cells, not previously subcultured, "[c]ell[s] grew exponentially on day 3 and 4 in culture. The characteristic exponential cell doubling time is approximately 30 hours. Cells appeared to be over confluent and went into lag phase after 6 days of culture" (Zhang Dec. September 2004, Appendix 9).

16. The Zhang Declaration discloses Figure 2, reproduced below:



"Figure 2 Characteristic growth curve of the 293 WCB P/N 08-00005, C/N001263" (Zhang Dec. September 2004, Appendix 9).

17. The Gallagher Declaration teaches "[w]ithout knowledge of seeding density, lag phase or doubling times for the cells used in that study, there is no way one can conclude that Huyghe *et al*. infected the cells between mid-log and stationary phase" (Gallagher Dec. 4 ¶ 6).

18. The Gallagher Declaration teaches

> At best, one can merely estimate the "phase" of the culture described in the Huyghe *et al.* reference, making certain assumptions and extrapolations, when infected at 2-2.5 days and 50- 60% confluency. If one assumes that Huyghe *et al.* seeded at a seeding density of approximately 1-3 x 10^4 cells/cm^2, and that the 293 cells employed by Huyghe *et al.* had a lag phase of approximately 24 hours and a doubling time of approximately 36 hours, then one can calculate the phase at 50-60% confluency as early log phase, certainly less than mid-log phase, using the following calculations:
>
> • Initial density (midpoint of assumption range) = 2 x 10^4 cells/cm^2
>
> • Growth period (2.5 days - lag time) = 1.5 days = 36 hours
>
> • With a doubling time of 36 hours, the cells population will double once, giving final concentration equal to 2 x 10^4 cells/cm^2 x 2 = 4 x 10^4 cells/cm^2, consistent with early log phase density

(Gallagher Dec. 4 ¶ 7).

19. Freshney,[2] which is discussed in the Gallagher Declaration, teaches that

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[2] R. Ian Freshney, *Culture of Animal Cells: A Manual of Basic Technique* 239-240 (1987).

> [t]oward the end of the log phase, the culture becomes confluent -i.e., all the available growth surface is occupied and all the cells are in contact with surrounding cells. Following confluence the growth rate of the culture is reduced, and in some cases, cell proliferation ceases almost completely after one or two further population doublings. At this stage, the culture enters the plateau (or stationary) phase

(Freshney 240).

20. Based upon Huyghe's 50-60% confluence data at 2.5 days (FF 8), a lag phase of 1-2 days (FF 9, 16), and doubling times of 1-1.5 days (FF 10, 12, 14, 16), the cells of Huyghe would reasonably be expected to have undergone one doubling at the time of infection. A second doubling, from 50-60% confluence to 100-120% confluence would reasonably place the cells in stationary phase, since the cells would have no further room to grow.

Principles of Law

"A rejection for anticipation under section 102 requires that each and every limitation of the claimed invention be disclosed in a single prior art reference." *In re Paulsen*, 30 F.3d 1475, 1478-79 (Fed. Cir. 1994); *see Karsten Manufacturing Corp. v. Cleveland Golf Co.*, 242 F.3d 1376, 1383 (Fed. Cir. 2001) ("Invalidity on the ground of 'anticipation' requires lack of novelty of the invention as claimed … that is, all of the elements and limitations of the claim must be shown in a single prior reference, arranged as in the claim.").

"[A] prima facie case of anticipation [may be] based on inherency." *In re King*, 801 F.2d 1324, 1327 (Fed. Cir. 1986). Once a prima facie case of anticipation has been established, the burden shifts to the Appellant to prove

that the prior art product does not necessarily or inherently possess the characteristics of the claimed product. *In re Best*, 562 F.2d 1252, 1255 (CCPA 1977) ("Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product.").

It is black letter law that "the PTO gives a disputed claim term its broadest reasonable interpretation during patent prosecution." *In re Bigio*, 381 F.3d 1320, 1324 (Fed. Cir. 2004). The court recognizes the fairness of reading claims broadly "before a patent is granted [since] the claims are readily amended as part of the examination process." *Burlington Indus., Inc. v. Quigg*, 822 F.2d 1581, 1583 (Fed. Cir. 1987). "Thus, a patent applicant has the opportunity and responsibility to remove any ambiguity in claim term meaning by amending the application." *Bigio*, 381 F.3d at 1324. Applying the broadest reasonable interpretation to claims also "'serves the public interest by reducing the possibility that claims, finally allowed, will be given broader scope than is justified.'" *In re Am. Acad. Of Sci. Tech. Ctr.*, 367 F.3d 1359, 1364 (Fed. Cir. 2004).

It is also settled that when reviewing decisions of this board the Court of Appeals for the Federal Circuit applies the "substantial evidence" standard. *See In re Gartside*, 203 F.3d 1305 1315 (Fed. Cir. 2000).

However, that standard does not apply to this board's review of an examiner's rejection. As stated in *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992), once an examiner presents a prima facie case of unpatentability, and the applicant responds with argument or evidence, "patentability is

determined on the totality of the record, *by a preponderance of evidence* with due consideration to persuasiveness of argument." (Emphasis added.)

Analysis

Claim 1

We begin by interpreting the claim phrase "mid-log phase of growth" (Claim 1). The Specification teaches that the "term mid-log phase of growth refers to the transition mid-point of a logarithmic growth curve" (Spec. 5, ll. 1-5; FF 1). The Specification also teaches that "mid log phase can be approximately defined as the point or interval within the log phase in which the cells are dividing at their maximal rate, and the increase in logs of cell number is linear with respect to time." (Spec. 22, ll. 14-17; FF 2.)

Thus, the Specification defines "mid-log phase" as an interval in which cells are dividing at their maximal rate, and where the increase in logs of cell number is linear. The Specification distinguishes "mid-log phase" from "late log phase," which is the "point or interval of time in which the rate of cell division has slowed" (Spec. 22, ll. 17-18; FF 3). We therefore interpret "mid-log phase," consistent with Appellants' Specification, as an interval in which cells are dividing at their maximal rate.

In balancing the evidence of record using this claim interpretation, we find that a preponderance of the evidence supports the Examiner's determination that Huyghe infected the producer cells "between mid-log phase of growth and stationary phase of growth" as required by claim 1.

In particular, Huyghe teaches the use of 293 cells (FF 7), the same cell type as that exemplified by the Specification (FF 4). Huyghe teaches that the cells were 50-60% confluent, and had been growing for two to two and

one half days at the time of infection (FF 8), which is consistent with the growth and infection at three days in the Specification (FF 5). The Specification noted that optimizing growth times was "well within one of skill in the art to determine" (Spec. 164, l. 12; FF 6).

The determination that Huyghe infected at "an interval in which cells are dividing at their maximum rate" is also supported by the other evidence of record. Kuchler discloses that lag phase is typically 24 to 48 hours, so at 48 to 60 hours, the cells of Huyghe are reasonably understood as necessarily being in the log phase interval in which cells are dividing at maximal rate (FF 11).

This is consistent with both the Zhang and Gallagher Declarations submitted by Appellants, in which Zhang teaches that 293 cells grew exponentially on day 3 of culture (FF 14-16) and Gallagher reasonably supposes that 293 cells had a 24 hour lag phase followed by at least one round of doubling, reasonably placing 293 cells at two to two and one half days in culture at an interval in which cells are dividing at a maximal rate (FF 17-18).

Further, based upon Huyghe's 50-60% confluence data at 2.5 days (FF 8), a lag phase of 1-2 days (FF 9, 16), and doubling times of 1-1.5 days (FF 10, 12, 14, 16), the cells of Huyghe would reasonably be expected to have undergone one doubling at the time of infection. A second doubling, from 50-60% confluence to 100-120% confluence would reasonably place the cells in stationary phase, since the cells would have no further room to grow (FF 19-20). In light of the reasonable interpretation of "mid-log phase" as defined by the Specification as discussed above, Huyghe's 293 cells, are reasonably found to have completed lag phase and one doubling in

log phase. The completion of a second doubling of Huyghe's 293 cells to 100-120% confluence would place the cells either into, or close to, stationary phase since "all the available growth surface is occupied and all the cells are in contact with surrounding cells" (Freshney 240, FF 19). Thus, Huyghe's cells are reasonably found to be in "mid-log phase" when infected.

We are not persuaded by Appellants' argument that the "Examiner has not met the burden of proof to establish inherent anticipation of independent claims 1 and 47 by Huyghe in light of Kuchler under 35 U.S.C. § 102(b) because the Examiner has neither shown that the characteristics of the present invention are necessarily present in the prior art, nor provided a basis to reasonably support such a determination" (App. Br. 7).

As discussed above, the preponderance of the evidence provided by the Examiner and Appellants supports the finding that the 293 cells of Huyghe are in "mid-log phase" at the time of infection (FF 1-20). We have carefully considered the evidence of the Specification, of Zhang and of Gallagher, and we conclude that this evidence shows that 293 cells at 2.5 days after seeding are necessarily in a state where they are dividing at a maximal rate and are, when at 50-60% confluence, one doubling away from stationary phase (FF 19-20).

We are not persuaded by Appellants' argument that the "MediaTech reference . . . stands for the proposition that one should use *at least* 70% confluent cultures to ensure that they are in log phase" (App. Br. 9). We understand the MediaTech reference as suggesting the use of 70% confluent cultures in the place of 100% confluent cultures to ensure that the cells are not in stationary phase rather than log phase (FF 13). MediaTech does not stand for the proposition the 50-60% confluent cultures are not in log phase,

but rather to use lower confluent cultures rather than fully confluent cultures to ensure log phase growth. Thus, to the extent that MediaTech teaches that 70% confluent cultures are log phase, this supports the Examiner's position that the 50-60% confluent cultures of Huyghe are also in log phase (FF 8, 13).

While we agree with Appellants that there is no "connection between L-M fibroblast cells in suspension and 293 cells grown on plates" (App. Br. 10), the preponderance of the evidence supports the Examiner's finding that 293 cells at 60 hours and 50-60% confluency are in log phase as discussed above (FF 13-20).

We are not persuaded by Appellants' argument that simply because Huyghe does not teach seeding densities, "the burden has not been properly shifted to the Appellants to provide absolutely substantiated evidence" (App. Br. 12). Viewing all of the evidence of record, including the disclosures that exponential growth for 293 cells occurs around two to three days after seeding (FF 14-18), that the 293 cells of Huyghe are 50-60% confluent (FF 8), and the interpretation of claim term "mid-log phase," the burden is properly shifted to Appellants to demonstrate that the 293 cells of Huyghe would not reasonably have been in a state of maximal growth or "mid-log phase." *See In re Best*, 562 F.2d at 1255("Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product.").

Claims 3 and 58

We agree with Appellants that the Examiner has provided no evidence that the 293 cells of Huyghe are "homogenous with respect to the phase of growth" as required by claims 3 and 58. While we recognize that "homogenous" does not require that the cells are synchronized, there is no evidence that the cells plated by Huyghe were derived from a single culture. The cells may have all been in log phase, but not homogenous, with some being derived from a plate that was 10% confluent and in early log and some from a plate that was 90% confluent in late log phase. Simply pointing out that the cells were 50-60% confluent at two and one half days after plating may show that the cells are, in gross, in mid-log phase, but this does not necessarily require or imply any homogeneity among the cells themselves. Thus, while we agree with the Examiner that the culture is in "mid-log phase," that finding does not require that the cells are "homogenous with respect to the phase of growth."

Claim 8

We are not persuaded by Appellants' argument that a teaching by Huyghe that 293 cells which were seeded for two to two and one half days (FF 8) does not satisfy a "comprising" claim limitation to attachment for "about 3 and [sic] about 24 hours" (*see* App. Br. 15). We note that "[t]he transition 'comprising' in a method claim indicates that the claim is open-ended and allows for additional steps." *Invitrogen Corp. v. Biocrest Mfg., L.P.*, 327 F.3d 1364, 1368 (Fed. Cir. 2003). We agree with the Examiner that the "claims do not require, as Appellant implies, that the period for attachment ends at hour 24 or that infection occurs at hour 24. The claim

merely requires between 3 and 24 hours of attachment prior to be[ing] infected" (Ans. 19).

Claim 9

We are not persuaded by Appellants' argument that claim 9 distinguishes between "recirculating the *culture* media *during* infection" and adding fresh media at the time of infection as taught by Huyghe (*see* App. Br. 16; FF 8). Claim 9 simply requires that "the culture medium is at least partially recirculated during the adenovirus infection step." We agree with the Examiner that when fresh medium is added with the virus during the infection step, this satisfies the requirement of claim 9 for partial recirculation (*see* Ans. 19).

Claims 13 and 31

In order to meet the "pharmaceutically acceptable" requirement of claims 13 and 31, the Examiner relies upon Huyghe's teaching that the "final virus pool from this step was dialyzed extensively against phosphate-buffered saline (PBS) supplemented with 2% sucrose and 2 m*M* $MgCl_2$" (Huyghe 1404, col. 2; FF 10).

We begin by interpreting the phrase "pharmaceutically acceptable" in light of the Specification. The Specification states that "[a]s used herein, 'pharmaceutically acceptable carrier' includes any and all solvents, dispersion media, coatings, . . . isotonic and absorption delaying agents and the like" (Spec. 113, ll. 13-16). The Specification specifically teaches that "human serum albumin per milliliter of phosphate buffered saline" as a pharmaceutically acceptable carrier (Spec. 114, ll. 17-18). The Specification teaches that the virus was filtered into phosphate buffered saline prior to inspection and labeling (*see* Spec. 167, ll. 25-30). The Specification also

teaches that sugars such as dextrose and lactose and magnesium salts may be included (*see* Spec. 114, ll. 23-30). The Specification exemplifies the use of sucrose and magnesium chloride (*see* Spec. 179, ll. 21-25). We therefore interpret the "pharmaceutically acceptable" formulations of the Specification as encompassing the use of solvents such as phosphate buffered saline, sugars such as sucrose, and salts such as magnesium chloride.

Having interpreted the scope of the claim limitation in light of the Specification, we are not persuaded by Appellants' argument that "the Examiner has not shown where Huyghe teaches that this solution is a pharmaceutically acceptable composition" (App. Br. 16). We conclude that the solution of Huyghe satisfies the requirements of a "pharmaceutically acceptable" composition in light of the Specification.

Conclusions of Law

(i) The Examiner did not err in finding that Huyghe teaches "infecting producer cells in the culture with the adenovirus, wherein the producer cells are infected between mid-log phase of growth and stationary phase of growth" as required by claim 1.

(ii) The Examiner did err in finding that Huyghe teaches that "the producer cells are essentially homogeneous with respect to the phase of cell growth" as required by claims 3 and 58.

(iii) The Examiner did not err in finding that Huyghe teaches that the producer cells are "allowed to attach to a culture surface for between about 3 hours and about 24 hours prior to infection with adenovirus" as required by claim 8.

(iv) The Examiner did not err in finding that Huyghe teaches that "the culture medium is at least partially recirculated during the adenovirus infection step" as required by claim 9.

(v) The Examiner did not err in finding that Huyghe teaches "a pharmaceutically acceptable composition" as required by claims 13 and 31.

B. 35 U.S.C. § 103(a) over Huyghe

The Examiner finds that

> Although Huyghe et al. do not teach the specific cell numbers to be plated, the number would be a subjective determination by one of ordinary skill based on many factors, such as the type of cell, the condition of the cells before plating, and the nature of the cell's division, ect. [sic] Therefore, it would be prima facie obvious for one skilled in the art to determine the appropriate number of cells to plate for each situation encountered.

(Ans. 7.)

Appellants contend that "Huyghe does not motivate or suggest to a person of ordinary skill in the art that one would determine seeding density in order to infect producer cells with adenovirus between mid-log and stationary phase of growth" (App. Br. 22). Appellants also contend that Huyghe "would not provide a person of ordinary skill in the art a reasonable expectation of success in infecting producer cells" (App. Br. 22). For claim 29, Appellants contend that "Huyghe does not teach or suggest adenoviral preparations that meet these limitations" (App. Br. 23).

In view of these conflicting positions, we frame the obviousness issues before us as follows:

(i) Did the Examiner err in finding that Huyghe renders obvious the specific seeding amounts of claims 10-12?

(ii) Did the Examiner err in finding that Huyghe renders obvious the specific purified adenovirus composition of claim 29?

Findings of Fact

21. The Specification teaches that "[i]t is well within one of skill in the art to determine that in other types of cell culturing systems, similar optimization of the seeding density for a particular system could easily be determined" (Spec. 164, ll. 9-14).

22. Huyghe teaches "purification of recombinant adenoviruses for use in human gene therapy trials and other research applications" (Huyghe 1403, abstract).

23. Huyghe teaches that the IZAC eluate was 98% pure (Huyghe 1411, col. 2, table 1). Huyghe also teaches that the IZAC eluate comprised 2.7×10^9 infectious units per ml (Huyghe 1412, Table 2).

24. Huyghe teaches that the IZAC eluate had a specific activity range of 88(±31):1, which represents a range of 57:1 to 109:1 (Huyghe 1412, table 2).

Principles of Law

The Examiner bears the initial burden of presenting a prima facie case of obviousness, and Appellants have the burden of presenting a rebuttal to the prima facie case. *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992). Appellants have the burden on appeal to the Board to demonstrate error in the Examiner's position. *See In re Kahn*, 441 F.3d 977, 985-86 (Fed. Cir. 2006).

Routine optimization of scientific variables by ordinarily skilled artisans that produce predictable variations are likely (i) the product not of innovation but of ordinary skill and common sense, and (ii) barred by 35 U.S.C. § 103. *See KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 417, 420 (2007); *Pfizer, Inc. v. Apotex, Inc.,* 480 F.3d 1348, 1371 (Fed. Cir. 2007).

"[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill," or unless application of the technique would produce unpredictable results. *KSR*, 550 U.S. at 417.

Analysis

Claim 10

Given that Appellants' Specification expressly teaches that seeding density is subject to "optimization" that is well within the level of one of skill in the art (FF 21), we find that in the absence of evidence of secondary considerations, these seeding densities are predictable variations which are the product of ordinary skill. *See Pfizer, Inc. v. Apotex, Inc.,* 480 F.3d at 1371. We agree with the Examiner that "since culturing cells for the purpose of propagating virus has been practiced since the 1950's, it is maintained that plating density would be knowledge generally available to one of ordinary skill in the art at the time the invention was made" (Ans. 20). Appellants have provided no evidence which demonstrates error in the Examiner's position.

Claim 29

Appellants state that "Huyghe does not teach or suggest adenoviral preparations that meet these limitations [of claim 29]" (App. Br. 23). However, the Examiner finds that

> Huyghe et al. teach a method of improving the quantity and/or purity of the recombinant virus obtained. Since improving quantity . . . is obviously a motivation for the ordinary artisan in the adenovirus art, it would have been prima facie obvious to one of ordinary skill to quantify any one of the properties listed to ensure a good yield of adenovirus

(Ans. 20-21).

We agree with the Examiner that since Huyghe was interested in obtaining large amounts of adenovirus for human gene therapy, Huyghe would have been interested in obtaining as large an amount of the virus as possible which was as pure as possible (FF 22). Huyghe teaches a virus whose titer and purity satisfy the requirements of element a) with an IZAC eluate comprised 2.7 x 10^9 infectious units per ml which falls between 1 x 10^9 and 1 x 10^{13} pfu/ml (FF 23) and f) with a purity of 98% which falls between 97 to 99% (FF 23). Huyghe also teaches element c) with a range of particle to PFU ratio which overlaps that of the claim, where the lower limit of the disclosed range falls within the 60:1 particle to pfu range of claim 29 (FF 24). "Whether the rejection is based on 'inherency' under 35 U.S.C. § 102, on 'prima facie obviousness' under 35 U.S.C. § 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products." *In re Best*, 562 F.2d at 1255.

Conclusions of Law

(i) The Examiner did not err in finding that Huyghe renders obvious the specific seeding amounts of claims 10-12.

(ii) The Examiner did not err in finding that Huyghe renders obvious the specific purified adenovirus composition of claim 29.

C. 35 U.S.C. § 103(a) over Huyghe, Graham and Leu

The Examiner finds that "Huyghe et al. do not teach infecting at late-log to stationary phase of cell growth" (Ans. 8). The Examiner finds that "Leu et al. teach a method of producing large quantities of virus by allowing uniform attachment of cells, growing the cells to late-log phase with medium replenishment to provide adequate cell nutrition and infecting the cells at late-log phase and harvesting the virus" (Ans. 8). The Examiner finds that Graham teaches "teaches infecting cells at 80-90% confluency with adenovirus" (Ans. 9).

Appellants contend that "a person of ordinary skill in the art would not be motivated to incorporate the methods to produce hepatitis A virus of Leu into the adenovirus production step of Huyghe to prepare adenovirus of the instant invention" (App. Br. 24). Appellants also contend that "there is no expectation of success in using a methodology designed to produce hepatitis A virus in human embryonic lung fibroblasts (MRC-5 cells) in the adenovirus production step of Huyghe, which deals with adenovirus grown in 293 cells" (App. Br. 24).

In view of these conflicting positions, we frame the obviousness issue before us as follows:

Did the Examiner err in finding that Huyghe, Leu and Graham render obvious infecting producer cells at late log phase?

Findings of Fact

25. Leu teaches that for production of hepatitis A virus "the cells are allowed to grow to late exponential (log) or early stationary phase" (Leu, col. 11, ll. 36-38).

26. Leu teaches that "[v]iruses which could be propagated in these host cells include, but are not limited to Hepatitis A, Varicella, Measles, Mumps, Rubella, Poliovirus, Herpes virus and Rotavirus" (Leu, col. 5, ll. 29-32).

27. Graham teaches that "[s]et up appropriate cells (HeLa or 293) 1 d prior to use, arranged to have the cells just at confluency when used. The actual split ratio depends on the cell type, but a good rule of thumb is about 6-8 60-mm dishes from each 150-mm dish of 293 cells" (Graham 117).

28. Graham teaches that "[a]fter determining the number of cells on a dish that is about 80-90% confluent, remove the medium and add appropriately diluted virus in 1 mL PBS^{2+}/150-mm dish" (Graham 117).

29. Graham teaches that "[w]hen the cytopathic effect is complete . . . harvest by scraping the cells off the plastic and centrifuging infected cells from the medium" (Graham 117).

Analysis

We think that Appellants have the better argument, since while Leu does teach growing hepatitis viruses in cells which are in late log phase (FF 25), Leu does not teach infection with adenovirus (FF 26). There is no particular reason, in the absence of evidence provided by the Examiner, to assume that all viruses grow best in the same phase of cell replication, whether lag, log or stationary phase. As Appellants note, adenovirus is a

double stranded DNA virus while hepatitis A virus is a single stranded RNA virus and these viruses differ in methods of release from cells, in modes and types of infection, and in physical structure from one another (*see* App. Br. 27).

We agree with Appellants that the cited prior art provided "no expectation of success in using a methodology designed to produce hepatitis A virus in human embryonic lung fibroblasts (MRC-5 cells) in the adenovirus production step of Huyghe, which deals with adenovirus grown in 293 cells" (App. Br. 24).

Conclusion of Law

The Examiner erred in finding that Huyghe, Leu and Graham render obvious infecting producer cells at late log phase.

D. 35 U.S.C. § 103(a) over Huyghe and Graham

The Examiner rejected claims 26-28 under 35 U.S.C. § 103(a) as being obvious over Huyghe and Graham.

The Examiner finds that "Graham et al teach that 5% sodium deoxycholate can be used to disrupt cells without disrupting adenovirus virions, see page 119. Therefore it would have been obvious to use deoxycholate or another detergent as an alternative method to lyse the infected cells" (Ans. 9).

The Examiner provides sound fact-based reasoning for combining Huyghe and Graham. As Appellants do not identify any material defect in the Examiner's reasoning, and we find none, we affirm the rejection of claims 26-28 for the reasons stated by the Examiner.

E. 35 U.S.C. § 103(a) over Huyghe, Garnier and Spier

The Examiner finds that "Huyghe et al. do not teach perfusion or the various culture systems recited [in the claims]" (Ans. 10). The Examiner finds that "Garnier et al. teach scale-up adenovirus growth using medium replacement for controlling glucose concentrations for improved virus yields in a bioreactor, see the material and methods section" (Ans. 10). The Examiner finds that

> Spier et al. review each of the various culture systems claimed, see the entire reference. One of ordinary skill in the art at the time the invention was made would have been motivated to use a conventionally applied culture system, described by Spier et al. in the method and system of Huyghe et al. and Garnier et al.

(Ans. 10.)

Appellants contend that "[t]he Examiner has not shown where any of these references, alone or when combined, teaches infection at mid-log phase" (App. Br. 35). Appellants also contend that "there would be no motivation to combine the very different goals of Garnier with the goals of the present application. Garnier concerns only the increased production of heterologous proteins using an adenovirus expression system and does not concern the production of adenovirus" (App. Br. 35).

In view of these conflicting positions, we frame the obviousness issue before us as follows:

Did the Examiner err in finding that Huyghe, Garnier and Spier render obvious producing the adenovirus in a perfusion reactor?

Findings of Fact

30. Garnier teaches that the "technological potential of adenovirus vectors (AV) in various applications such as . . . gene therapy . . . currently

gives rise to growing interest from biotechnologists. All of these applications will require the production of large quantities of either recombinant proteins or AV stocks" (Garnier 145, col. 1).

31. Spier teaches that "[p]erfusion is a long-established technique in cell culture (since 1912) . . . The current use of perfusion is in response to the need to maintain high cell densities in a unit volume" (Spier 190).

32. Spier teaches methods of growth including microencapsulation (Spier 201), multiplate culture systems (Spier 206), and microcarrier culture systems (Spier 208-209).

33. Spier teaches that "[s]caling-up from small laboratory flasks (static or spinner) in a unit rather than multiple process has always been a prime objective for cell culturists. Initially it was to meet the demands of virologists both for research and the manufacture of vaccines" (Spier 181).

Analysis

We have already affirmed that Huyghe satisfies the infection at mid-log phase requirement of claim 1 for the reasons discussed above.

Huyghe teaches production of adenovirus for gene therapy (FF 22). Garnier teaches that given the use of adenovirus in gene therapy, large amounts of virus will need to be produced (FF 30). Spier teaches a variety of known methods of scaling up production to meet the demand of virologists (FF 31-33).

We conclude that the Examiner has set forth a prima facie case that claim 4 would have been obvious to the ordinary artisan in view of Huyghe, Garnier, and Spier. *See KSR*, 550 U.S. at 417 ("[w]hen a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a person

of ordinary skill can implement a predictable variation, § 103 likely bars its patentability.")

We are not persuaded by Appellants' argument that Garnier "teaches conditions to increase production of heterologous proteins at the expense of virus production, thereby teaching away from the claimed invention" (App. Br. 35-36). Like our appellate reviewing court, "[w]e will not read into a reference a teaching away from a process where no such language exists." *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co*., 464 F.3d 1356, 1364 (Fed. Cir. 2006). Garnier clearly teaches that whether protein production or virus production is preferred depends upon the goals of the producer, but that both may be desirable (FF 30).

Conclusion of Law

The Examiner did not err in finding that Huyghe, Garnier and Spier render obvious producing the adenovirus in a perfusion reactor.

SUMMARY

In summary, we affirm the rejection of claims 1, 8, 9, 13, and 31 under 35 U.S.C. § 102(b) as being anticipated by Huyghe. Pursuant to 37 C.F.R. § 41.37(c)(1)(vii)(2006), we also affirm the rejection of claims 14-25, 38, 47, 49, 51-57, and 59-62, as these claims were not argued separately.

We reverse the rejection of claims 3 and 58 under 35 U.S.C. § 102(b) as being anticipated by Huyghe.

We affirm the rejection of claims 10-12 and 29 under 35 U.S.C. § 103(a) as being obvious over Huyghe.

We reverse the rejection of claims 2 and 50 under 35 U.S.C. § 103(a) as being obvious over Huyghe, Graham and Leu.

We affirm the rejection of claims 26-28 under 35 U.S.C. § 103(a) as being obvious over Huyghe and Graham.

We affirm the rejection of claim 4 under 35 U.S.C. § 103(a) as being obvious over Huyghe, Garnier and Spier. Pursuant to 37 C.F.R. § 41.37(c)(1)(vii)(2006), we also affirm the rejection of claims 30, 39-46, and 48, as these claims were not argued separately.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv)(2006).

AFFIRMED-IN -PART

cdc

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